

**This Page Is Inserted by IFW Operations
and is not a part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- **BLACK BORDERS**
- **TEXT CUT OFF AT TOP, BOTTOM OR SIDES**
- **FADED TEXT**
- **ILLEGIBLE TEXT**
- **SKEWED/SLANTED IMAGES**
- **COLORED PHOTOS**
- **BLACK OR VERY BLACK AND WHITE DARK PHOTOS**
- **GRAY SCALE DOCUMENTS**

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

In re: Walsh et al.
Appl. No. 09/689,430
Filed October 12, 2000

APPENDIX B

1. Get primary Rat hepatocytes from Dr. Lola Reid's lab

①. count the cells: $3.5 \times 10^6/\text{ml}$

②. Talk with Dr. Reid and Ed

a. special media: HDM

b. plate medium HDM + 10% FBS

culture medium with or without FBS

c. Collagen I help ~~adhere~~ the cells adhere to the bottom of dishes

Collagen II can also induce quick differentiation of the cells besides adhere

d. if using collagen insert, plate the cells in well of insert

e. plate 5×10^5 cell/well in 6-well plate the cells will be confluent in 3-5 days however, depending on how frequent you feed the cells

[I found it is too much cells if plating 5×10^5 in one well of 6-well plate)

① 2×10^5 / well in 6-well plate

② 5×10^5 _____

③ 2×10^5 / well in 6-well plate with
collagen I or VI insert

④ 5×10^5 / well in 6-well plate with
collagen inserts

for the sake of saving ~~FBs~~ with Vif in FBs
I will use FBs

Infect Rat Hepatocytes

The cells in common plastic plates adhered well.

The wells with 5×10^5 cells are almost

confluent, so I chose the 2×10^5 to use in
common plastic 6-well plate to use

suck off the media

wash the wells with media

Add the media with virus (free of FBs)

to each well, plate at incubator for

1.5 h. swirl the plate each 15 minutes

The cells adherent to the bottom of the
well during the infection

(1) (2) (3)
(4) (5) (6)

① 200 μ l VAAV/DLZ
② 100 μ l
③ 10 μ l
④ 1 μ l
⑤ 0.1 μ l
⑥ 200 μ l VAAV/DLZ

(200 μ l viruses sol contain about
 $2-3 \times 10^{10}$ physical particles)

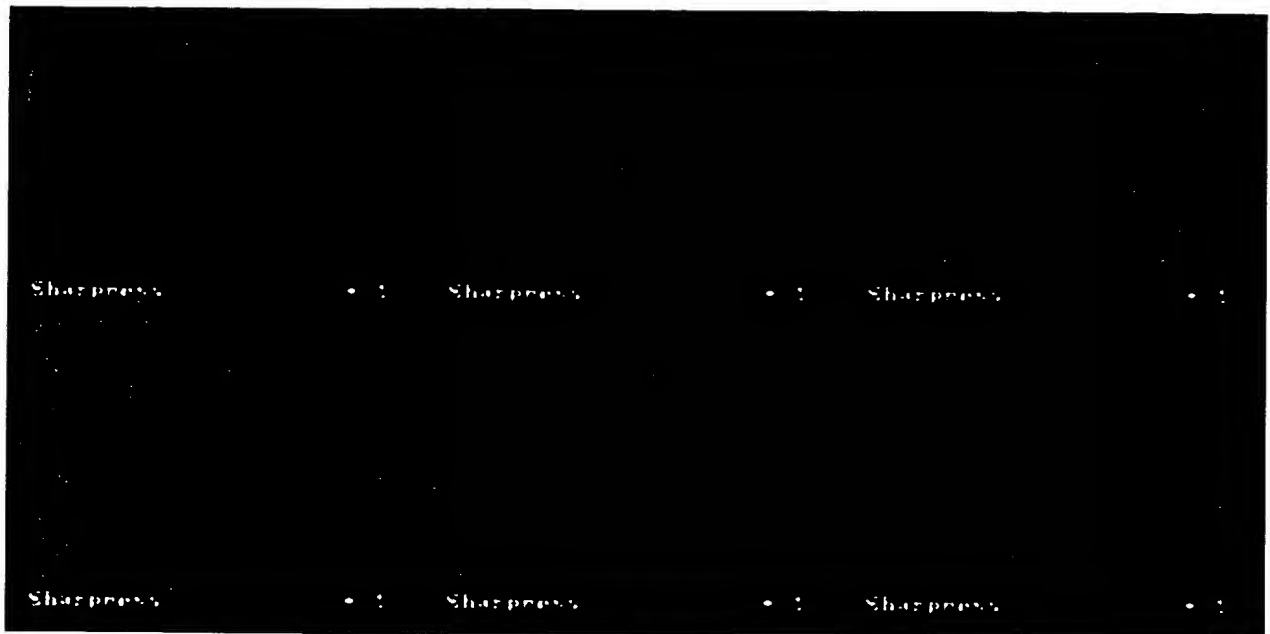
Add media (no serum) to 1 ml of each

observe the Rat-Hepatocytes / EGFP under
UV filter with blue color. all of the cells
even the negative control (well 6) turned
green.

③ Dr Bagnell in microscopy facility confirmed
all the cells had sth with green fluorescence
on Monday

③ Diem's said, actually all of the primary
Hepatocytes, Pog. human. Rat. mouse secret sth

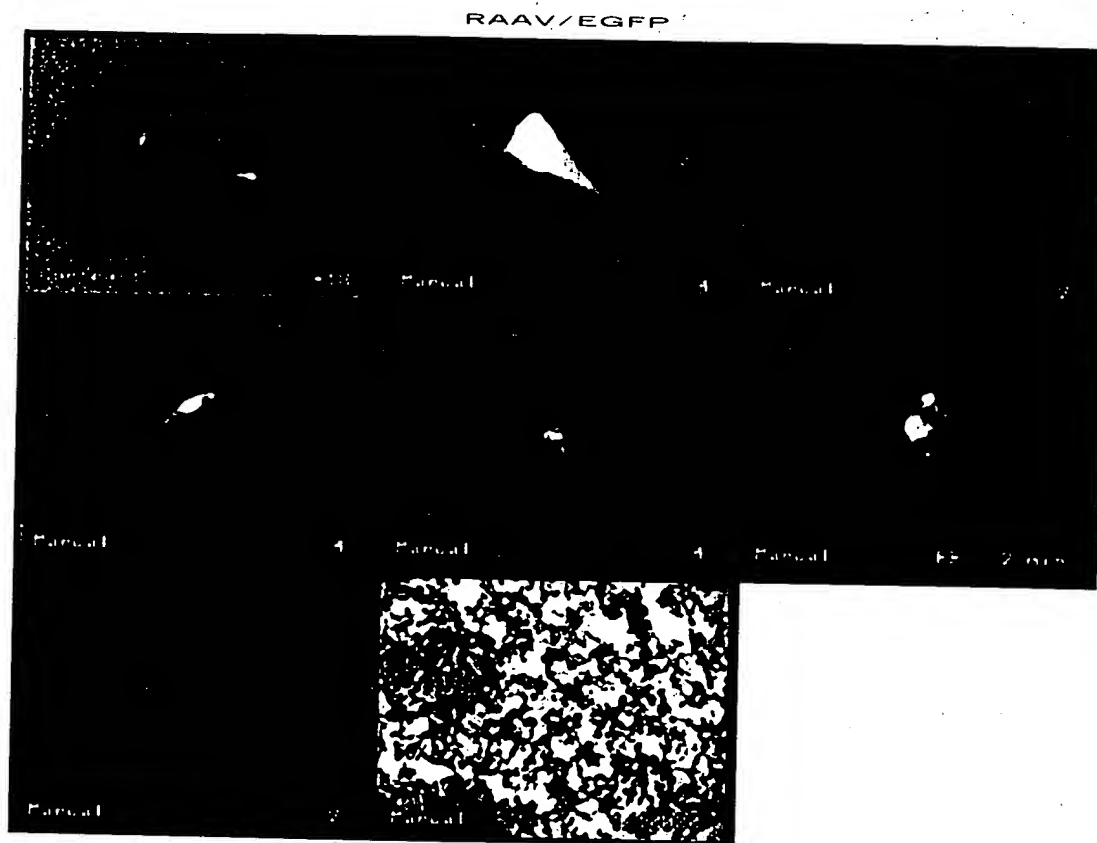
rAAV/EGFP-Rat.heptocyte



like EGFP, turn the filter to yellow
one can give some help

④ Did as Dennis taught

Took the pictures



1	2	3
4	5	6
7	8	

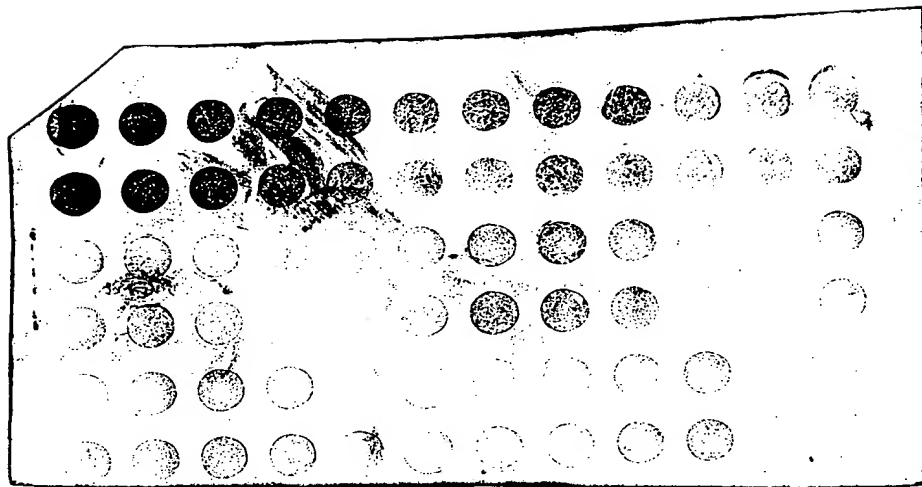
None green cells in well 6
few in well 4~5

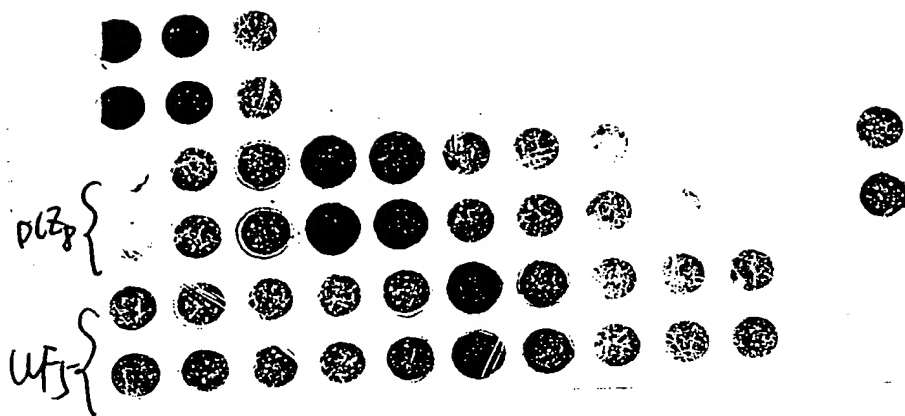
1-6: picture from of
cells from well 1-2

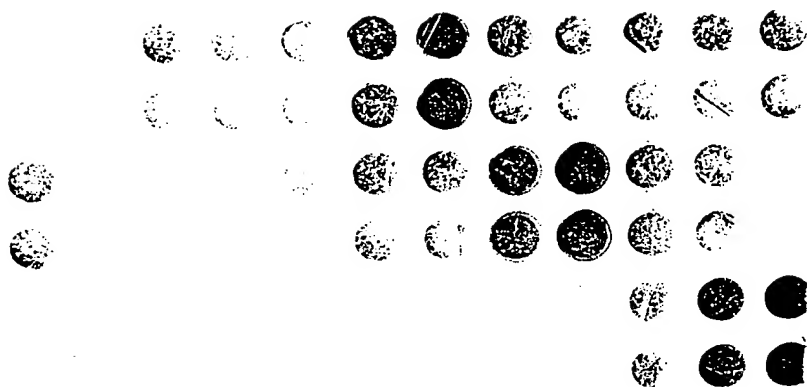
7: cells of well 6

8: cells under common

light







⑤ Talk with Chris, not sure whether these green cells are Hepatocytes instructed me to do other Hepatocyte cell lines

⑥ Coatest of Hepatocytes / DLZ6

Definitely some Flu activity from HepG2 cells
 about very low

TABLE OF ABSORBANCE VALUES

FILE:

TITLE: FILE 1

AT 04:45 PM

	1	2	3	4	5	6	7	8	9	10	11	12
	0.05 IU/ml	0.024	0.012	0.006	0							
Standard Curve	0.445	0.483	0.406	0.289	0.213	0.036	0.046	0.045	0.045	0.046	0.045	0.044
	0.494	0.371	0.388	0.253	0.214	0.042	0.045	0.044	0.046	0.045	0.036	0.041
	0.301	0.237	0.228	0.195	0.210	0.037	0.239	0.184	0.045	0.045	0.045	0.046
	0.245	0.160	0.242	0.203	0.201	0.196	0.227	0.182	0.046	0.045	0.047	0.045
duplicate	0.351	0.308	0.278	0.249	0.204	0.049	0.045	0.046	0.047	0.047	0.050	0.044
standard	0.358	0.306	0.291	0.257	0.210	0.047	0.046	0.045	0.047	0.047	0.048	0.044
duplicate	0.222	0.235	0.214	0.214	0.223	0.042	0.224	0.221	0.048	0.047	0.047	0.045
sample	0.216	0.244	0.221	0.220	0.226	0.224	0.225	0.224	0.049	0.047	0.051	0.048
	293	HepG2	WBFL44	rat	293	HepG2	WBFL44	rat				
	day 5	day 5	day 5	primary cells day 3	9	9	9	9				

⑦ Appt: Day 5's sample's

HepG2 / F8: 96.9 / 114.4 / 109.9

WBFL44 / F8: 129.9

293 / F8: > 150

HepG2 / 0: 148.9 / > 150

9 > 150

0 > 150

1:160 uCRP (6.3%) 102.9/101.9

1:320 uCRP (3.1%) 125.8/118.9

there are about some activity between 3-6%

compared to Results, it is similar. The reason of the low expression of F8 perhaps are a weak promoter, even with help of Enh.

② low infectious efficiency of the viruses with the cells. (see DLZ6 infecting primary Rat Hepatocytes)

③ In accurate titer of viruses

→ not enough viruses

I am going to make more viruses purified by 2 times ultra-spin.

Re-titered by radio-avine dot blot.